FTS CDC EPO

Moderator: Denise Korzeniowski
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12:00 pm CT

Coordinator: Good afternoon and thank you all for holding, this is the operator. And at this time, I just like to inform you that you are in a listen-only mode until we open for questions and answers.

Also this call is being recorded. If you have any objections, so please disconnect.

At this time, I would like to turn this call over to Ms. Roberta Bartholdi.

Thank you, ma'am, you may begin.

Roberta Bartholdi: Good day. Welcome to our teleconference, Avoiding Diagnostic Dilemmas and Routine Rabies Testing.

This is Roberta Bartholdi, the QA Manager and Laboratory Practice's Consultant at the South Carolina Department of Health and Environmental Control in Columbia, South Carolina.

Today's teleconference is being hosted by the South Carolina Department of Health and Environmental Control.

A few program notes before we begin the program.

CDC, our partners and our presenters wish to disclose they have no financial interests or other relationships with the manufactures of commercial products, suppliers of commercial services or commercial supporters. This presentation will not include any discussion of the unlabeled use of a product or a product under investigational use.

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If time permits, the end of the program will be opened up for questions. Remember, you are on a listen-only line. We cannot hear you; you can only hear us.

If you experience any problems with the line during the conference, please press star-0. This will signal the attendant that you were having a problem.

If the program experiences technical difficulties, please do not hang up. Stay on the line until the issue is resolved.

Again, welcome and thank you for joining us.

We have over 60 sites from across the United States listening to this teleconference.

Today's speaker is Lillian Orciari.

The speaker's rabies' experience started with employment at the Vermont Department of Health. But since 1991, she has been performing research activities at CDC in several areas related to rabies diagnostic testing, including development of protocols for antimortem samples by RT-PCR, extraction of RNA from Formalin-fixed tissues, adaptation of formalin-fixed DFAs with a commercially-available conjugate, molecular typing of rabies viruses.

She also interacts with state and regional laboratories to resolve diagnostic problems through implementation of the standard DFA protocol, and provides assistance in confirmatory DFA testing.

It is my pleasure to introduce to you and to welcome our speaker, Lillian Orciari.

Lillian Orciari: Thank you, Roberta.

Today, we'll be talking about avoiding diagnostics dilemma and routine rabies testing.

And if we go to our second slide, we'll see that since the development of the first rabies vaccine, accurate and timely diagnosis of rabies inspections in animals has been essential to prompt and successful host-exposure treatment of humans.

The next slide, diagnostic test highlights the fact that the direct (unintelligible) antibody test is the gold standard by which all other diagnostic tests for rabies are evaluated.

If we review the second slide - actually the third slide, we'll see a review of the DFA with applications to rabies virus.

Rabies virus replicates cytoplasm of infected of neuron. When brain impressions are made, a thin layer of cells are coated onto slides. Within the cells are inclusions, collections of the rabies viral protein or antigen. When the rabies virus conjugate, antibodies to rabies virus labels of fluorescein, added to the slides and incubated an antigen-antibody complex form which is labeled with the fluorochrome, and the specific viral antigen will (unintelligible)..

Next slide.

Avoiding diagnostic dilemmas involved several factors. Some of these are the sample, the condition, correct ID, position sampling, and avoiding cross-contamination.

The Reagent, the conjugate needs to be pre-titrated before use and other reagents evaluated.

Close attention needs to be made to the expiration date, and then plan appropriately when ordering reagents.

Technical expertise is important, and training should be provided on a regular basis, and review made of inexperienced personnel test.

Interpretations of the results are important, and all rabies CSA test slides need to be reviewed by two leaders.

Safety is always important, but not the topic of this presentation.

In Slide Number 6, we will discuss that all persons working in the rabies laboratory should have pre-exposure immunization and demonstrate a minimum neutralization titer of 1 to 5.

Rabies neutralization titer should be checked every two years. And the safety requirements are addressed in the BMBL fourth edition.

The video for removal of brains from animals may be obtained from NLTN.

In this particular slide, we'll discuss the number one step in avoiding cross-contamination. And this is at a point of necropsy or sample collection.

We need to store all clean instruments, containers, lab maps and slides in a clean area. You should work with only one specimen at a time. You need to use a clean set of instruments for each specimen, label each sample immediately with a unique number, change gloves between samples, and store all samples and animal carcasses until test had been reported.

It's important to decontaminate the necropsy table after processing each specimen. And of course, we must always decontaminate all instruments by autoclaving and clean them before reusing.

Next slide.

We still must emphasize that extreme care must be taken to ensure the proper numbering and labeling of each samples and to avoid cross-contamination because mistakes made at necropsy cannot be easily resolved by repeat testing and the performance of confirmatory test.

Methods such as isolation and cell culture for mounting (occultation), and RT-PCR may amplify a rabies virus contaminant.

Next slide.

Rabies travels along the nerve tracks from the side of infection to the brain. Usually, the virus is widespread in the brain tissue, but it may be more focal or unilateral -- that is infecting one side of the brain in large animals.

Across section through the brain stem, we'll test the maximum number of ascending and descending nerve tracks.

Next slide.

This slide demonstrates the sampling of tissues for preparation of brain impression.

The national standard protocol requires testing a complete cross section through the brain stem. And that can be taken from the areas of the medulla, pon or midbrain, as seen in the upper right.

It also requires testing of the cerebellum, including the vermis right and left lateral lobe, seen in the lower pictures.

Testing of the hippocampus on the right side of the next slide will historically require for rabies diagnosis with histologic stain. But testing of the hippocampus is no long required. If the cerebellum is unavailable for testing however, testing the right and left hippocampi may be substituted for testing of the cerebellum.

Although the brain stem is the tissue most reliably found positive, inclusion of the cerebellum or the hippocampus samples in the test give an additional confidence in making a rabies diagnosis.

Sometimes, unacceptable samples are received. And this can be deteriorated or decomposed samples. Sometimes, they're easily distinguished by loss of structural characteristics, or they may display some coloration (unintelligible)side or defecated.

However, we must emphasize that negative results should never be reported on deteriorated samples. The test report should state the rabies could not be ruled out due to this condition of the sample.

Formalin-fixed tissues cannot be tested by the standard DFA test. Chemical-cross linking of the proteins interferes with antigen binding. Other tests such as the formalin-fixed DFA test and an immunohistochemical test protocol can be used on these samples. And arrangements can be made at CDC to perform this type of testing if a formalin-fixed sample is received in your laboratory.

Next slide.

The slide, what you'll see now demonstrates the minimum number of slides required for the standard protocol. This would be one set of duplicate impression across section through the brain stem and duplicate impression through the cerebellum including the vermis right and left lateral lobe.

Slides are air dry before acetone fixation.

Preparations of the touch impression are very important. Impressions or smears should be made, then blotting of the slides on paper towels can remove excess brain tissue. The thick impression or smears might drip rabies conjugates and make interpretations difficult due to non-specific fluorescing.

Thick smears are also more likely to wash off in the rinse.

Acetone fixation is another important step. Each set of slides from a sample should be fixed in a separate container for each sample set of slides.

Slides should be fixed with fresh acetone for each set of slides, and fixed at minus 20-degree for one hour to overnight (unintelligible).

The positive and negative control slides should be fixed at the same time, but again in separate containers, (aminofluorescein).

Samples must be tested with two different anti-rabies conjugates.

Conjugates are pre-filtered to low protein binding filters, 0.45 filters attached to syringes, and the conjugate is added directly onto the slides.

The first three drops is discarded and then the conjugate is added first to the positive control slide, then to the test slide, and last to the negative control slide to ensure that specific antibodies are not absorbed by the filter in the initial drop.

In addition, the negative control slide is added last because the associated fluorescein will be noticed on the negative slide if it is a problem in the test.

Again, next slide will emphasize that two different conjugate preparations must be used on every test. If Fujirebio Diagnostics, Center for Anti-Rabies Conjugate is used, the conjugate paired with that conjugate must be either the Chemicon Light Diagnostics DFA Reagent 5100, or a polyclonal product Chemicon Light Diagnostics Rabies Polyclonal Conjugate Reagent 5199.

Chemicon DFA Reagent 2 should not be paired with the Fujirebio Diagnostic Conjugate because both conjugates contain identical monoclonal antibodies.

Why use two different conjugates? Because commercial hyperimmune polyclonal conjugates are broadly reactive that may have some non-specific reactions to other than other agents than rabies.

Monoclonal antibodies by definition are two single epitope. Monoclonal antibody conjugates contain two or three monoclonal antibodies to highly conserve rabies and protein epitope.

If use optimal working delusion, the commercial conjugates detect all rabies various variance found in the United States.

If there is a lack of reactivity, which one of the monoclonal antibodies in a conjugate, there'll be a reduction in the reactivity observed.

This should not be a problem if the optimal working delusion has been predetermined. However, in some cases, if a problem in detection occurs with one of the conjugate, the additional conjugate paired with that should be able to detect the rabies virus antigen.

Next slide.

After incubating with the conjugates, the slides are rinsed with a PBS-wash bottle and later placed into separate containers for washing. Again, each specimen set of slides needs to be separated in a separate wash container. This can be a Copeland jar staining dish; (unintelligible). And slides are rinsed twice for 3 to 5 minutes in PBS.

After the PBS rinses, there's no need to do a water rinse, slides can be blotted or air dried and mounted with on coverslip.

When mounting the coverslip the slide, you should use a 20% glycerol, tris-buffered mounted. And the mounting should be added to the coverslip, not the slide. And then either the slide can be inverted onto the coverslip or this coverslip can be inverted onto the slide. But the important thing is that the mounting is added to the coverslip to avoid the possibility of cross-contamination.

The standard protocol has specific criteria for evaluating slides. Slide-staining intensity is graded from 4 plus to 1 plus. Antigen distribution is also graded from 4 plus to 1 plus, whereas 4 plus, massive infiltration small and large antigen in almost every area of the slide.

Three-plus antigen distribution, we see inclusions of varying size and shape in every field, but the number of inclusions may vary but they're still quite numerous.

Plus two inclusions are of varying size and shape intense to 50% of the field, and most fields contains only a few inclusions. The criteria is important to be familiar with particularly because the (unintelligible) on the report ability of the test without repeat testing is at the levels of 2 plus.

One plus inclusions - 1 plus distribution has inclusions of varying size and shape in less than 10% of the microscopic field. And in this case, only a few inclusions are found per field.

The next slide demonstrates a positive sample with 4-plus antigen distribution.

Go to next slide, we see a negative sample with no specific rabies virus antigen detected.

Let's review the interpretation of results.

Tests are complete or can be recorded if the positive and negative control slides give their appropriate result. If the sample tissues were in satisfactory condition and adequate amounts of tissue were tested, then the test can be considered complete or incomplete based on the observed pattern sustaining.

A test of negative if no specific staining is observed in the test slides with the two different anti-rabies conjugates. The slide is positive, clearly positive with both anti-rabies conjugates if there is 3-plus to 4-plus staining and 2-plus to 4-plus antigen distribution.

Slides need to be repeated that is the tests are incomplete.

On the next slide, if there are typical inclusion but they're less than 10% of the examined field, or in some cases, there are typical inclusions but they're in greater than 10% of the field. But each field only contains maybe one or two inclusions per field.

In some cases, the inclusions might look typical, but the intensity of the fluorescein is weak -- that is less than expected, less than 3 plus.

In other cases, the inclusions are atypical. That means the staining might be 4 plus, but the inclusions are regularly sized or uniformed in texture. In other cases, there maybe fluorescing bacteria that can map small amounts of rabies-specific staining, or there can be particulate or free-forcing with (lightmap) mass small amount of rabies-specific staining.

Results should also be repeated when there are discrepancies or differences between two-week results with two different reagents, or two different meters.

The next slide demonstrates a case when a test should be repeated. When we see (it starts) fluorescent staining, and in this particular case, it's unclear whether it's in one plane or not, and it just - when there is less than 1% - less than 10% staining like this, it's difficult sometimes to resolve whether this is positive and it needs to be re-tested.

In other cases such as this one, we see not apple-green fluorescent; you see sort of a bright-golden staining. This should not be a problem. No rabies, virus antigen is detected in this slide. The inclusions are also atypical, they're atypical in morphology, and they're atypical in staining intensity and staining color.

In this particular case, 150 people received needless rabies PEP. The laboratory should have performed a confirmatory DFA test if they were at all concerned about this particular slide before repeating - before reporting the result.

Another slide with atypical bacterial-like staining is in your next slide. You can see the following slide has atypical staining intensity and atypical morphology. And if you look to the arrow, you can see that this is very focal on this slide. So we have three reasons to repeat this particular test. We have atypical staining intensity, atypical morphology, and we have less than 10% fluorescent.

Now we'll review repeat and confirmatory testing. All incomplete DFA test must be repeated with two conjugates and at least one specificity control reagent, a negative control conjugate. Slides are remade from the original brain tissue, sordid autopsy at necropsy in the same manner as previously described. That is in this case, we need three impressions or smears made from each tissue.

If we look at the principles of the specificity control, the specificity control conjugate is a non-rabies - contains non-rabies antibody which are labeled with FITC. And they should be of the same isotype and prepared in the same animal as the diagnostic conjugate.

Each of the commercially-available conjugates has a negative control conjugate or specificity control regent that can be purchased to be paired with that particular reagent. And each must be diluted to the same concentration and milligram per ml after diagnostic test reagent.

In review of the principle, if we have a positive test and there's rabies antigen on the slide, and we add diagnostic conjugate, we'll see a specific antigen antibody complex formed, and we'll see specific fluorescein.

If no - if rabies antigen is present, and we add the specificity control conjugate, the negative control conjugate, we'll have no antigen antibody complex formed and we'll see no specific rabies fluorescein. There'll be no inclusions because there are no antibodies to rabies in the conjugate.

If we review the next slide and no rabies antigen is present on our test slide, and we test with the diagnostic conjugate, we'll see no antigen antibody complex formed, no rabies-like inclusion, and we add the specificity control reagent to the slide, the specificity control reagent of course doesn't have antibodies to rabies, no antigen antibody complex formed, no specific fluorescein, no rabies viral inclusions will be observed.

In some case -- next slide -- we will see. Sometimes, the non-rabies antigen is of bacterial origin. In these cases, when we add the diagnostic conjugate, we see a non-specific antigen antibody complex. In some case, this is due to non-specific binding of the FC receptor. And then we will observe atypical inclusion with a diagnostic reagent, and the specificity control reagent will react in the same manner. And we will observe non-specific antigen antibody complex binding from the FC receptor in the inclusion flow of atypical.

In other cases, non-specific fluorescent is not due to FC receptor binding, but to other reasons. Sometimes it may be a technical problem to a washing or reagent problem disassociated (fluoroscope), and in other cases, it maybe a reaction due to charged particle.

In these cases, we may see non-specific fluorescent and atypical staining with both the diagnostic conjugate and the specificity control reagent, the negative control conjugate.

However in some cases, under these conditions, the reaction maybe random, and we may see some non-specific fluorescent in either of the two test impressions. This is the most difficult perhaps type of non-specific fluorescent to address, and one has to look for specific rabies-like inclusions to be able to rule out or diagnose rabies. And we'll discuss some ways to interpret these slides, and additional testing would be required.

Okay. Now that we've reviewed the reaction with the specificity control, let's discuss the confirmatory DFA test.

We talked before about the fact that we need to repeat the test, testing with two anti-rabies conjugates, and a specificity reagent paired with one of the two conjugates.

If in conjugate one, the diagnostic conjugate, we have no specific rabies-like inclusion staining, in conjugate two - with conjugate two, the test is negative, there are no specific rabies virus antigen detected, and the specificity control reagent stained impressions we also have no specific rabies virus inclusions detected, the slide is - the test is negative.

If on the other hand, we have positive stain with the first conjugate and positive staining with the second conjugate with inclusions that are typical of rabies, and the negative control, the specificity control reagent is negative, then the test can be reported out as positive.

Here in the next slide, we repeat the test and Conjugate 1 shows a negative result with both impressions, Conjugate 2, we see non-specific staining of bacteria, and with conjugate - with negative control conjugate, the specificity control reagent paired with Conjugate 2 we also see non-specific (progressing) bacteria, then the test can be reported out as negative.

If in the next set of slides we see that Conjugate 1 is negative, but Conjugate 2 demonstrates rabies-like inclusion, and the specificity control the agent could - paired with that reagent is negative, then we should consider the test positive. But it needs to be confirmed, that is it needs to be sent to a reference lab for confirmation and alternative testing.

Another set of slides should have been concluded - included. And I'm going to describe that even though you don't have that set - that slide.

If there is fluorescent indistinguishable from specific rabies virus antigens in all three (route) -- that is in the impression stained with Conjugate 1 or stained with Conjugate 2 and those stained with specificity control reagent paired with one of the two conjugates, so that the test conjugates and the control reagent all have fluorescence that's indistinguishable from rabies antigen, the test should be reported as non-diagnostic and it needs to be sent to a reference lab for confirmation and for alternative test to rule out or confirm rating.

The next slide, so in summary, standard protocol requires adequate sampling of brain tissue from the brain stem -- that is a cross section through the pons,

medulla or the mid-brain, and from the cerebellum including the vermis right and most lateral lobes.

Acetone fixation should be for a minimum of 1 hour at minus 20 degrees to overnight. And each sample set of slides needs to be in a separate staining container. Positive and negative control slides should also be fixed at the same time as test slides.

The protocol requires the use of two anti-rabies conjugates in every test. Slides should be rinsed and two changes of the PBS for 3 to 5 minutes. But again, to avoid cross contamination, separate containers are required for each set of slide.

Coverslip are mounted two slides with the 20% glycerol tris-buffered mounting, and 2 meters are required to evaluate the two set of slides.

There is repeat testing of weekly positive and inclusive test using rabies - using a rabies conjugate and specificity on negative control conjugate.

So again, we need to repeat the weekly positive and inclusive result with the two anti-rabies conjugates, and at least one specificity control reagent paired to one of the - one or the other of the two conjugates.

In conclusion, the protocol for post-mortem diagnosis of rabies in animals, the minimum standards of protocols for the United States includes steps to avoid cross-contamination to maintain sensitivity and specificity, and criteria's for evaluation of the tests result, including confirmatory testing of repeat DFA and permission to a reference laboratory.

It requires compliance of the US Laboratories to these standards. And there are pre-testing procedures to avoid cross-contamination during brain removal and preparation of the brain impression. But these are not specifically addressed from the protocol, although these issues were addressed in the CDC videotape removal of brain from the animal survey diagnosis.

Additional training materials, slides or an outlet need to be developed to familiarize the laboratory in which color intensity and morphology of specific rabies inclusion.

Alternate confirmatory test needs to be investigated. The direct rabies from immunohistochemical test drip seems to be a highly sensitive test for rabies virus antigens detection and a possible confirmatory test.

A discussion of this test was published in the last issue of EID last month. And this can be obtained online.

RT-PCR as a sensitive method, however limitations include the condition of the sample and RNA, primer match and the protocol used. And until true universal primers are developed to amplify all rabies virus (experienced), usefulness of RT-PCR as a diagnostic tool will be limited.

The National Working Group on rabies diagnosis should meet to address some of these issues as well as discuss the problems associated with compliance.

Although 115 of the 121 laboratories performing rabies diagnostic surveyed in 2003 were familiar with the standard protocol, only 45 of the 115 -- that's 39% -- performed the minimum standard protocol as written. In response, the

NLTN and CDC rabies training courses were held in January and March 2004 and January 2006 to acquaint laboratorians with the standardized protocol.

Thank you very much.

And now if anyone has any questions, I'd be happy to address these. Thank you.

Coordinator: Thank you.

And at this time, if you do have a question, please press star-1 on your touchtone phone. And you'll be asked to record your first name for identification. Thank you.

Once again at this time, please press star-1 on your touchtone phone.

Thank you. One moment.

Roberta Bartholdi: Thank you.

We will now take your questions.

The operator will give you instructions.

Coordinator: Thank you.

Once again at this time, please press star-1 on your touchtone phone and please record your first name so you may be announced.

One moment.

Roberta, your line is open.

Roberta Bartholdi: Thank you.

We'll now take your question.

(Robbie Lopez): Hi, this is (Robbie Lopez).

Coordinator: Thank you ma'am, we can hear you.

(Robbie Lopez): I was just wondering about the slide that's entitled "Inclusion"…

((Crosstalk))

(Robbie Lopez): It's one of the last slides on Page 7.

Lillian Orciari: Yes?

(Robbie Lopez): And I was just wondering about this test called, "the Direct Rabies Immunohistochemical Test." I was wondering if you could tell me something about it.

Lillian Orciari: Yes. It's actually immunohistochemical test. And I see procedure. And it - what it - what actually how it's performed is there's a cocktail monoclonal antibodies that's biotinylated, and then avidin-biotin substrates added and it's actually performed just like a routine IHC-type procedure.

If you're interested in this type of test, you can contact the CDC for training. At the present time, the test is in it, I believe in most of data testing. And if

you are interested and want to receive training, please contact Dr (Charles Rupert) at the CDC. And I'd be happy to give you that information and you're welcome to email me at lao0@cdc.gov. And I'd be happy to forward your request for training to Dr. (Rupert).

Coordinator: Thank you, my next question comes from (Lon). Your line is open.

(Lon): Yes. My question is you recommended through these slides on a minus 20 freezer. In the past 10, 15 years ago, we used to place the slide on room temperature. What is the need to put them around there minus 20? Can simply put them at room temperature for one hour?

Lillian Orciari: No, (Lon). Hi, how are you doing?

(Lon): We're doing fine.

Lillian Orciari: That's good.

No, the standard protocol is that the slides need to be at the jumpsticks at minus 20-degree. The standard protocol is just that we're trying to have one national protocol so that all laboratories will do the acetone fixation and do the test in exactly the same way. And this is very important if were to compare results from laboratory-to-laboratory.

The reason why fixing slides…

(Lon): Okay.

Lillian Orciari: …at room temperature is not appropriate is that we're concerned about denaturing the protein. And I think it's better stick with the standard protocol and fix your slides at minus 20.

Thank you.

(Lon): I do have another question though.

((Crosstalk))

Lillian Orciari: Hey, (Lon), do you have another question?

(Lon): Yes I do.

Woman: It works fine.

(Lon): You also indicated that this rabies ways should be out okay about - as by 60 minutes. Normal protocols call for 30 minutes. What - will that work or do we need to increase that for 60 minutes?

Lillian Orciari: We were just interested in increasing the time to 60 minutes because of other agents other than rabies. And so, there's a concern about other agents, perhaps even the 60 minutes is even enough for BFC for us. But that was the reason we're increasing the time.

(Lon): Okay, well thank you very much.

Roberta Bartholdi: Operator, are there any more questions queued?

Coordinator: Thank you. I do have a question from (Shin-tei), your line is open.

(Shin-tei): Okay, yes. Actually, we have checked with Chemicon Company, they do have a specificity control conjugate. But we checked with Fujirebio Diagnostics, they don't have specificity control conjugate.

My question is, if we have non- specific bindings from Fujirebio Diagnostic Conjugate, do we still need to use that specificity control conjugate from Chemicon?

Lillian Orciari: That's a very good question. And we actually talked to someone from Fujirebio at the same time when we were having (NLTN) training course.

The conjugate, Fujirebio Diagnostics Conjugate, those particular antibodies are the same as the antibodies in the Reagent 2, the DFA 2 reagent from Chemicon. So the - if the antibodies themselves are reacting nonspecifically with the Chemicon product, they should also react nonspecifically with the Fujirebio products since they contain the same identical antibodies.

Fujirebio however is interested in preparing a specificity control reagent which would go with their conjugate which I think would be worthwhile. And we're looking forward to that product in the future.

(Shin-tei) Can I have another question?

Lillian Orciari: Yes, I guess if the monitor allows it.

Coordinator: Yes, go ahead sir.

(Shin-tei): Okay. Actually we (contact) with the CDC Rabies Laboratory, we are interested to have a training there. We just want to know the cost for that

training - for that kind of training. Is there (unintelligible) - is there vacancy for 2006?

Lillian Orciari: We just had the NLTN training course in January. Are you asking about specific bench training at CDC?

(Shin-tei): Yes.

Lillian Orciari: Then again, if you need training at CDC, you need to call or email Dr. (Charles Rupert), and I'll be happy to again forward that email. Or if you contact me at CDC after the teleconference, I'll be happy to try to arrange something.

(Shin-tei): I mean like a sponsorship, we can apply and - or any (glance) we can apply for that kind of training?

But now in Massachusetts, we don't have money to support this kind of training. So that's our problem.

Lillian Orciari: Well we just have the NLTN training course, you know, in January for that purpose. I think again, you need to talk to Dr. (Rupert), but I don't know if funding would be available. I think that would have to come from the state. But you need to talk to the rabies chief to arrange for that.

(Shin-tei): Okay, thank you very much.

Coordinator: Thank you.

And my next question comes from (Ralph). Your line is open.

(Ralph): Hi Lillian. I got a question about testing the cerebellum?

Lillian Orciari: Yeah.

(Ralph): Is it actually necessary to test the complete cross section of the cerebellum or can we take like people want to send us just one side of the brain…

Lillian Orciari: Uh-hum.

(Ralph): …and have that just the one side.

Lillian Orciari: Well…

(Ralph): And the complete cross section of the spinal cord.

Lillian Orciari: I think the complete cross section through the brain stem is important.

(Ralph): Right.

Lillian Orciari: As far as the cerebellum go, if you read the standard protocol, it will say, if you are unable to test the complete cerebellum -- that is both sides -- that you can test more sections from the side you do have.

(Ralph): Right.

Lillian Orciari: And that will be satisfactory.

I would rather test both sides personally, but the protocol does say that you can just make more slides from the half you do have. However, the protocol does say that you must test the cross section through the brain stem, and a test

cannot be diagnosed if a complete cross section through the brain stem is not available for testing. So you could report out a positive result, but you cannot report out as a negative result. The negative result would have to be reported as a disclaimer that would say, rabies cannot be ruled out due to lack of…

(Ralph): Plate sample or something like that?

Lillian Orciari: Yeah.

(Ralph): Okay, thank you very much.

Coordinator: Thank you.

Just a reminder at this time, if you would like to ask a question, please press star-1, record your first name for identification.

One moment.

Thank you.

Once again at this time, if you would like to ask a question, please press star-1, record your first name for identification.

Thank you. One moment.

Thank you, (Chuck), your line is open.

(Chuck): Hello Lillian, it's (Chuck) for (RNT).

I've just don't see it on the slides, that may have been in other material that was distributed. But could you tell us where we would find the National Protocol?

Lillian Orciari: Yes. And I thought they would actually put that up on the Website for us. And so yes, you can get to the link from the CDC Rabies Website. And if you do that then it is located at - and I can give you that in a second here. And it should be at http://www.cdc.gov/ncidod/dvrd/rabies/Professional/publications/DFA_diagnosis/DFA_protocol-b.htm. And I'm sure that NLTN will be happy to email that to all the participants if they do not have it upon their slides.

(Chuck): That will be up on there.

Okay, thank you. Thank you.

Coordinator: Thank you.

(Steve), from Washington, your line is open.

(Steve): Yeah, this is (Steve).

And two questions. One is on the compliance test slides. It's a little disconcerting on the 39% of that (met) protocol. Is there any updated numbers on that and what other steps to increase that?

And then the other question is, are anything on working with Department of Ag for a lot the animal testing? Are they incorporating the standard of protocol also?

Thank you.

Lillian Orciari: We need to actually do a new survey now after the additional training courses, how many laboratories are now performing the standard protocol as written. And from our experience, laboratories are very interested in complying and participants of the training courses were very encouraging and that they wanted to go back to their laboratories and make changes.

I don't know if they able to do that, if there are some supervisors that will allow them to make those changes, but I think we expect that if we were to take a survey today, I assume that we would see - and I hope we will, and we do intend do that survey in the future.

The rest of your questions, do you mind repeating that?

(Steve): With regarding that, I believe like a lot of the labs that test-like animal exposure only I think with Department of Ag, do they follow this protocol? Is there any move for them to do a standardized protocol also?

Lillian Orciari: Yes there is, and we do have communications with those laboratories. And many of the people from those laboratories also attended some these training courses or have had bench training at CDC recently.

((Crosstalk))

Coordinator: Thank you.

And once again at this time just a reminder, if you would like to ask a question to please press star-1 and record your first name.

Thank you. One moment.

Thank you. At this time I have no questions.

Roberta Bartholdi: Okay, thank you.

I have a question here. Do control slides need to be made at the time of testing or ahead of time?

Lillian Orciari: That's a very good question, Roberta. Actually, the control slides can be made in advance. And if they're made in advance of course we don't want to fix those because we want use those as control for the acetone fixation.

And you can make your control slide your negative and your positive control slide in advance, dry them for 15 to 30 minutes, and then store in the freezer either at minus 20 degrees or at minus 70 degrees. And those can be stored at minus 20 degrees for up to one month, and can be stored for six months in advance at minus 70 or minus 60.

Roberta Bartholdi: Thank you for that response.

Operator, are there any more questions? We have time for one more.

Coordinator: Thank you, ma'am. I do have one more question from (Chasis) in Rhode Island.

Your line is open.

(Chasis): Actually, I'm sorry but I have two questions. One regarding rabies variant monoclonals. Should state labs be doing that? And if so how do you go about doing that? Or you have any protocols available?

And second is related to PCR. How can our lab get into gear to be doing PCR for confirmatory test as needed? We do have the capacity and capabilities.

Lillian Orciari: Okay. The answer I think to both of the questions is if you would like training, I think if you want to do variant monoclonal antibody testing in your laboratory, I think you could request training at CDC and we'll happy to train you in both antigenic typing with monoclonal antibody typing and RT-PCR. Again, arrangements need to be made.

We did cover some of this in the rabies training course which was that CDC. And monoclonal antibodies can be purchased. They're commercially available like Light Diagnostic Chemicon. And there is a protocol and we'll be happy to provide with the protocol for doing this.

(Chasis): Can I have a follow up please?

Lillian Orciari: Yeah, we'll be happy to send you the protocol.

(Chasis): Sometimes, money is not available to send somebody for training. Is there a way to get the protocol that we can try on our own with consultation from your group there?

Lillian Orciari: We don't really recommend testing without some kind of training. But you, you know, you're able to do that if you like to purchasing the commercial product. There are regional laboratories in your area I know that the New York State Department of Health also is doing monoclonal antibody typing

and perhaps arrangements could be made and perhaps it would be such a big (drive to accompany).

There is the Massachusetts State Department of Health, but I don't know if they are currently doing on monoclonal antibody typing but they have in the past.

So you may have local laboratories that do this type of testing, and you could go to a regional laboratory for some training before trying to do the monoclonal antibody typing in your lab on you own. Because I think it's important when starting a new test to at least have appropriate training, and you have to be aware of the fact that with monoclonal antibody typing you need to titrate the antibody with known rabies viruses before actually using them for your test. And you need to know what kind of reaction patterns you would expect to see. Sometimes it maybe a little bit difficult to determine whether it's a positive reaction or not with the monoclonal antibody because there can be decreased of reaction in the (wealth). Thank you.

(Chasis): Thank you for your suggestion, I really appreciate it. I will call (Tye Marky) or (Bob Rod) and see if they would be willing to. Maybe they are listening to this.

Lillian Orciari: I think they are, and I'm sure they might be able to assist with you since you're their area.

(Chasis): Even in the past, they've always been very kind, so I will follow up on that.

Lillian Orciari: I think that would be wise. Thank you.

Roberta Bartholdi: Operator, are there anymore questions queued?

Coordinator: Thank you, not at his time.

Roberta Bartholdi: Okay. Thank you very much.

I would like to thank Ms. Orciari and also to let you know that she will answer any of your questions by e-mail if you can have a question after this presentation is over.

Please e-mail your question to meoffice@nltn.org. Again, that e-mail address is meoffice@nltn.org.

Again, I would like to remind you all that listening to our program, to register and complete an evaluation form by April 14. When you have completed the registration and evaluation form you will be able to print your Continuing Education Certificate. The directions for these are on your confirmation letter and general handout.

Documenting your participation helps us to continue to bring high quality cost-effective training program in a variety of formats.

This concludes our program. Our next teleconference will be on April the 19th. The topic is "Being Prepared, Having a Continuity of Operation's Plan."

The co-sponsors of today's program would like to thank our speaker Lillian Orciari, and thank you all for joining us. I hope that all of you will consider joining us for future programs and that you will make the National Laboratory Training Network your choice for laboratory training.

From the South Carolina Department of Health and Environmental Control in Columbia, South Carolina this is Roberta Bartholdi. Good day.

END